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Queen primer pheromone affects conspecific fire ant (*Solenopsis invicta*) aggression

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Abstract Monogyne fire ant, *Solenopsis invicta*, colony workers are territorial and are aggressive toward members of other fire ant colonies. In contrast, polygyne colony workers are not aggressive toward non-nestmates, presumably due to broader exposure to heritable and environmentally derived nestmate recognition cues (broad template). Workers from

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both monogyne and polygyne

fire ant colonies execute newly mated queens after mating flights. We discovered that monogyne and polygyne queens have a remarkable effect on conspecific recognition. After removal of their colony queen, monogyne worker aggression toward non-nestmate conspecifics quickly drops to merely investigative levels; however, heterospecific recognition/aggression remains high. Queenless monogyne or polygyne worker groups were also not aggressive toward newly mated queens. Queenless worker groups of both forms that adopted a monogyne-derived newly mated queen became aggressive toward non-nestmate workers and newly mated queens. We propose that the powerful effect of fire ant queens on conspecific nestmate recognition is caused by a queen-produced recognition primer pheromone that increases the sensitivity of workers to subtle quantitative differences in nestmate recognition cues. This primer pheromone prevents the adoption of newly mated queens (regulation of reproductive competition) in *S. invicta* and when absent allows queenless workers to adopt a new queen readily. This extraordinary discovery has broad implications regarding monogyne and polygyne colony and population dynamics.

Introduction

"Phenotype matching" (Holmes and Sherman 1982) appears to be a reasonable model for nestmate recognition in ants. Chemical cues residing on an intruding individual's cuticle are detected by a resident worker's antennae, and the detected cues are compared with the experience-based neural template of the resident (Gadagkar 1985; Breed and Bennett 1987; Vander Meer et al. 1989). If the cues match the template then no aggression occurs. If the cues do not match the template then aggression ensues (Wilson 1971; Holldobler and Michener 1980; Vander Meer and Morel 1998). Thus, the standard way to investigate nestmate recognition is through aggression bioassays. Olfactory inputs to worker labels can include heritable "discriminators" from the queens and/or workers, as well as cues acquired from environmental (e.g., food, soil) sources (see reviews in Breed and Bennett 1987; Holldobler and Carlin 1987). The leaf-cutting ant, *Acromyrmex octospinosus*, uses only environment-derived cues (Jutsum et al. 1979); the acacia ant, *Pseudomyrmex ferruginea*, uses only heritable discriminators (Mintzer 1982); and the fire ant, *Solenopsis invicta*, uses a combination of environmental and heritable cues (Obin and Vander Meer 1988). All potential cues can be distributed to other colony members through grooming and trophallaxis, creating a colony "Gestalt" (Crozier and Dix 1979; Vander Meer and Morel 1998).

Normally, polygyne colonies that occupy multiple adjacent nests (polydomy) display little or no intercolony discrimination when compared to monogyne colonies (Holldobler and Wilson 1977; review in Breed and Bennett 1987; Morel et al. 1990). However, some population-level recognition has been reported (Traniello 1982; Halliday 1983; Stuart 1987; R.K. Vander Meer, unpublished data). The generally accepted proximate ex

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planation for the association of polygyny with reduced nestmate recognition is the discriminator variability hypothesis. The reduced recognition in polygyne populations has been suggested to be a consequence of increased within-colony genetic variation in queen and worker-derived recognition cues that results from the presence of multiple queens, multiple worker matriline/patrilines, and wider access to environmental cues (H61ldobler and Wilson 1977; Breed and Bennett 1987; Stuart 1987; Vander Meer and Morel 1998). In this scenario, worker templates, based on queen or worker discriminators and/or the environment, become sufficiently "broad" to accommodate the colony recognition cues of non-nestmates (Vander Meer and Morel 1998).

The relative importance of the cue sources for small colonies of the carpenter ant, *Camponotus floridanus*, has been determined to be queen discriminators > worker discriminators > environmental cues (Carlin and Hdldobler 1986). In contrast, when environmental cues (e.g., food, rearing conditions) were held constant for *S. invicta* colonies, queen discriminators were not found to play a significant role in colony-level recognition (Obin and Vander Meer 1989a) and worker discriminators appeared to dominate the heritable cues.

The present study examines the influence of monogyne and polygyne *S. invicta* queens on conspecific aggression/recognition by comparing aggression bioassay results for queenright and queenless colony situations. Instead of investigating the contribution and role of queen discriminators to colony nestmate recognition cues (Obin and Vander Meer 1989b), e.g., heritable cuticular lipids, we report the first evidence for the release of a queen-produced primer pheromone that has a profound effect on worker recognition/aggression toward conspecific workers and newly mated queens (NMQs).

Methods

Monogyne and polygyne *S. invicta* populations

Both monogyne and polygyne *S. invicta* populations can be found in the vicinity of Gainesville, Fla. Specific *S. invicta* field sites were determined to be monogyne or polygyne by sampling representative colonies throughout the site and evaluating them using the following criteria. An *S. invicta* population was designated monogyne if all of the following characteristics were found: low mound density (<100/hectare); well-developed, large nests; polymorphic characteristics of colony workers (Greenberg et al. 1985); no more than one inseminated queen found; and a high level of intra-specific aggression (Morel et al. 1990). An *S. invicta* population was designated polygyne if it met all of the following criteria: high density (>150/hectare) of small mounds; more than one inseminated queen found per colony; and low levels of intra-specific aggression (Morel et al. 1990).

Collection and maintenance of queenless monogyne and polygyne worker groups

Queenless worker groups from a particular social form were collected as follows. Beakers or Erlenmeyer flasks (250-500 ml) were coated on the upper inside lip with Fluon (ICI Americas, Exton, Pa.), which prevented the exit of worker ants that entered the

container. If the mound surface was soft, the containers were simply pushed into the mound and excited workers would crawl up the sides of the container, fall in, and accumulate over time. If the mound was hard, a shovel was used to dig it open and the container was placed into the area where the ants were most excited. Generally, workers carrying brood moved down into the nest and the workers crawling up the sides of the containers were responding defensively. No brood or alates were collected in this manner. The containers were maintained in the mounds until adequate numbers of workers had been collected. Each queenless worker group was immediately brought back to the laboratory, weighed, and the number of workers adjusted by weight to approximately 700 mg (ca 1,000 individuals). These groups were set up in porcelain trays (29x18x5 cm), whose inner sides were coated with Fluon to prevent escapes. Each worker fragment was provided with a nest cell, comprised of a test tube (15 cm longx 2.2 cm diameter) half filled with water and plugged with a cotton ball. The unfilled half of the test tube was covered with red cellophane to simulate a dark nest environment. The worker colony fragments were maintained in the laboratory with water from their test tube cell and a diet of crickets and sugar/water (1 M sucrose) provided in another test tube plugged at the end with a cotton ball.

Source of NMQs

S. invicta NMQs were collected from late May and summer mating flights in the vicinity of the USDA laboratory in Gainesville, Fla. This collection area is primarily composed of monogyne colonies; therefore, the NMQs were expected to be monogyne derived. To ensure that the NMQs collected were not from a previous day's mating flight, the area of collection was searched for queens in the morning prior to the projected mating flight and all queens found were eliminated. If a prior search and removal sortie was not possible, then only NMQs that were found walking on the surface of the collection site were taken. NMQs were weighed (Ohaus GA200-D; Ohaus Corp., Florham Park, N.J.) and only those >14 mg (indicative of monogyne derivation; Keller and Ross 1993, 1995) were used in the experiment.

Queenright *S. invicta* and *S. richteri* colonies

Queenright monogyne *S. invicta* colonies were obtained from the Gainesville, Fla., area by rearing colonies from NMQs or by excavation of nests. After finding the queen, the colony was isolated by floating out the workers and brood by standard procedures (Banks et al. 1981). *S. richteri* colonies were collected from Lee County, Miss., by the excavation method described above, circa 15 months prior to tests. These colonies were designated pure *S. richteri* based upon gas liquid chromatographic analysis of venom alkaloid constituents and cuticular hydrocarbons (Vander Meer et al. 1985; Ross et al. 1987). All colonies isolated after excavation were placed in rearing trays (52.Ox39.Ox7.5 cm) and provided with petri dish nest cells (diameter 14.0 cm) and cotton-stoppered water tubes (Obin 1986). Colonies were reared on crickets and 10% sugar water absorbed onto wads of tissue. Colonies from NMQs were started by setting up the NMQs claustrally in test tube "nuptial" chambers. These colonies were given sugar water and crickets after the first workers eclosed. As the colonies grew, their nest trays were changed appropriately. Rearing temperatures were maintained between 21 and 28°C, and the light:dark cycle was variable.

Aggression bioassay

The nestmate recognition bioassay (Obin 1986) measured aggression in the context of nest defense. Individual workers, "intruders," were allowed to walk onto a pair of forceps and were then allowed to walk off the forceps into trays containing "resident" colonies. Intruder ants were discarded if they showed disturbed behavior prior to their introduction into the resident colony. Obser-

Table 1 Behavioral units and aggression scores used to assess nestmate recognition (see Obin and Vander Meer 1988)

Score	Behavioral response
9	Immediate lunge, grab and stinging
8	Opponent surrounded and "held" in mandibles; appendages pulled/bitten off; eventual stinging
7	Opponent held (as in No. 8) but released; biting; abdomen curling (stinging position), but no stinging
6	As in No. 7, but no abdomen curling
5	Alarm (running, abdomen elevation and vibration) and recruitment
4	Mandible gaping; rapid antennation; "sidling" (maintaining a lateral orientation to and slowly circling opponent)
3	Rapid antennation with antennae extended for more than 2 s
2	Antennation for less than 2 s; if mobile, opponent is followed slowly for several centimeters; if opponent stationary, worker stops
1	As in No. 2, but opponent does not induce following or stop

variations were made by an observer wearing a particle or surgical mask to minimize disturbance by exhalation on the ants. Individual intruders were removed from resident colonies immediately after testing. Additional intruder introductions into any one resident group were spaced at least 15 min apart to allow resident workers to recover from the potential disturbance of the previous introduction. Bioassays were conducted at 26–28°C.

The resident colony response to each intruder was scored on a (1–9) scale of increasing aggressive behavior (Table 1; Obin and Vander Meer 1988). We recorded only the most aggressive response observed during an intruder's interaction with ten resident ants, because of individual variability in response and to guarantee independent sampling. Resident colony responses for each treatment were computed from these individual introduction scores. The ten interactions usually required less than 10 min to complete for worker intruders. The observations were terminated if the highest level of aggression was observed before the tenth interaction. Significantly different treatment pairs were identified by the Mann-Whitney -U-statistic (Sokal and Rohlf 1988), because assumptions of ANOVA could not be met (Obin 1986).

The effect of queen removal on conspecific worker aggression

The objective of this series of experiments was to determine if the presence of the queen influences conspecific worker/worker aggression. (a) Five queenright monogyne and five polygyne colonies were collected from the field as described above. Each polygyne colony was set up with five of their queens and 2.1 g of workers and brood. The monogyne colonies were set up with their queen and 2.1 g of brood and workers. The following day, conspecific nestmate recognition was measured for colonies within each group of five using the aggression bioassay described above. This

provided baseline aggression data for each queenright colony. (b) Ten queenless subunits from each queenright colony (monogyne and polygyne) were prepared, one with and the other without brood. Each subunit contained about 0.6 g of workers (ca 800) and those with brood also contained 100–200 pieces of brood. Twentyfour hours later, aggression bioassays commenced on the colony subunits and continued once a week for 5 weeks (aggression levels measured at 1, 7, 14, 21, 28, 35 days). (c) At the end of the 5week period, conspecific nestmate recognition of the queenright (monogyne and polygyne) parent colonies was re-evaluated. (d) In addition, the heterospecific recognition capabilities of the monogyne and polygyne 5-week old queenless subunits (broodless and with brood) and the parent queenright colonies were evaluated by

introducing queenright *S. richteri* worker intruders into a4.experi-;^ mental colonies

The effect of an adopted queen on conspecific worker nestmate recognition

The objective of this series of experiments was to determine if the adoption of a queen by queenless *S. invicta* workers would have an effect on their conspecific nestmate recognition capabilities. Queenless *S. invicta* workers readily adopt newly mated queenw (R.K. Vander Meer and L.E. Alonso, unpublished data). Polygyne workers were collected from the field and used within a few hours as nestmate recognition intruders. The aggressive behavior of the resident workers was recorded according to the graded scale in Table 1 and the method shown above. (a) Polygyne-derived worker intruders were introduced into queenless monogyne worker subunits ($n=15$) that had previously adopted an NMQ (now queen right). (b) Polygyne-derived worker intruders were introduced into polygyne worker groups that had been queenless before adopting at least one newly mated queen. For bioassays (a) and (b), the polygyne- and monogyne-derived worker groups had maintained their adopted queens for about 4 months prior to the nestmate recognition/aggression bioassays.

The influence of the queen on worker aggression toward conspecific NMQs

This series of experiments was conducted to determine if the presence of the colony queen influences conspecific worker aggression toward NMQs. Newly mated *S. invicta* queens were collected as described above. Within 3 h of collection, NMQs were (a) introduced into monogyne queenright colonies ($n=16$) as described for the introduction of workers. The aggressive response of workers to the introduced NMQs was recorded based on the aggression scale detailed in Table 1. (b) In a separate experiment, NMQs were introduced into monogyne queenless worker subunits ($n=14$) collected directly from the field. The queenless subunits had been without their queen for at least 4 weeks prior to NMQ introduction. The aggressive behavior of resident workers toward the intruder NMQs was recorded. (c) Under similar conditions, NMQs were introduced into queenless monogyne worker subunits ($n=15$) that had previously adopted an NMQ (now queenright; R.K. Vander Meer and L.E. Alonso, unpublished data) and the aggressive behavior of the workers was recorded. Worker interactions with NMQ intruders were not counted while the NMQ was motionless (see Results)

Results

The effect of the queen on conspecific worker/worker aggression/nestmate recognition

Workers from queenright monogyne colonies are known to be territorial and highly aggressive toward non-nestmate conspecific intruders, whereas polygyne-derived workers are not aggressive toward members of other conspecific colonies, polygyne or monogyne (Morel et al. 1990). We discovered that monogyne *S. invicta* colonies that lose their queen become non-aggressive toward conspecific workers (See Table 1 for aggression scores; queenright: median=7.5, interquartile range (IQR)=1.5, $n=20$; queenless for 4 weeks: median= 1.5, IQR=3, $n=20$; $U=30.000$, $P<0.0001$). To determine the time dependency of this dramatic change in conspecific aggression, we measured the drop in aggression with increasing time after being made queenless.

The introduction of non-nestmate *S. invicta* worker intruders into queenright monogyne colonies elicited high levels of aggression (median=9.0, IQR=2.0, n=20; see Fig. 1, time=0, and Fig. 2). Aggression scores above six generally lead to the death of the intruder, which was expected for the territorial monogyne social form of this ant. In contrast, after monogyne colony workers had been queenless either with or without brood for only 1 day, their aggression scores were significantly lower than the queenright results (broodless: median=7.0, IQR=5.5, n=20; U=111.000, P=0.016; with brood: median=6.5, IQR=6.0). The aggression levels of queenless monogyne colony workers, with or without brood, continued to decline until they were not significantly different (>28 days) from their queenless polygyne colony worker counterparts (Fig. 1; broodless: U=142.500, P=0.1199, n=20, t=28 days; with brood: U=193.000, P=0.8498, n=20, t=28 days). The presence or absence of brood did not affect the decrease in worker aggression for queenless monogyne colonies. We found no significant differences in aggression scores for monogyne queenless workers with or without brood throughout the duration of the experiment (greatest difference at 28 days; broodless median=1.0, IQR=2.5 vs with-brood median=1.0, IQR=0.0; U=143, P=0.1231, n=20). Aggression levels decreased logarithmically over time for both queenless monogyne worker types (Fig. 1; broodless, $Y = -2.3841 \log x + 5.905$, $R^2 = 0.827$, $P < 0.001$; with brood, $y = -1.8911 \log x + 5.053$, $R^2 = 0.797$, $P < 0.001$). Aggression levels increased slightly with time for polygyne queenless colonies, whether they were broodless or with brood (Fig. 1; broodless, $y = 0.013x + 0.980$, $R^2 = 0.531$, $P = 0.063$; with brood, $y = 0.021x + 0.954$, $R^2 = 0.594$, $P = 0.042$); however, there were no significant differences between the two categories of queenless polygyne colonies (e.g., time period t=35 days had the greatest difference; broodless: median=1.0, IQR=0.0 vs median=1.0, IQR=0.0 with brood: U=169.5, P=0.409). We introduced heterospecific workers, *S. richteri*, into the queenless *S. invicta* colonies that were no longer aggressive (day 35; Fig. 1) toward conspecific worker intruders to determine if being queenless generally lowered worker recognition/aggression capabilities. *S. richteri* intruders elicited maximal levels of aggression from queenright mother colonies or queenless monogyne-derived worker residents, with and without brood (Fig. 1: aggression level: median=9, IQR=0.0, n=13). Similar results were obtained when *S. richteri* intruders were introduced into polygyne queenright mother colonies or polygyne-derived broodless or with-brood queenless worker groups (aggression level: median=9, IQR=0, n=13). Lastly, aggression scores within each group of monogyne and polygyne queenright mother colonies were determined at the end of the experiment to test whether or not the laboratory environment was responsible for the lowered monogyne worker aggression after queen removal. There was no significant difference in queenright aggression levels before and after the experiment (Fig. 1; t=0 and 35 days, U=179, P=0.570, n=20).

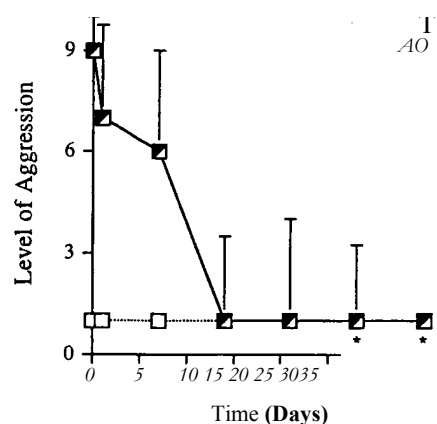


Fig. 1 Plot showing the effect of queen removal on fire ant worker conspecific recognition over time. Recognition was measured using an aggression bioassay (see Table 1 for the graded aggression response scale). The median and 1/2 IQR are shown for each specified group. Both monogyne and polygyne social types were evaluated [open squares queenless polygyne workers without brood, half-shaded squares queenless monogyne workers without brood, open triangle the aggressive response of monogyne workers that had been queenless for at least 35 days to heterospecific *Solenopsis richteri* worker intruders (offset from 35 days for visibility), open circle worker aggressive response of the queenright parent colonies toward each other 35 days after the queenless subcolonies were assembled]. The aggression levels for monogyne worker groups and polygyne workers groups were significantly different for all time periods except those marked with an asterisk. The presence of brood did not affect the results

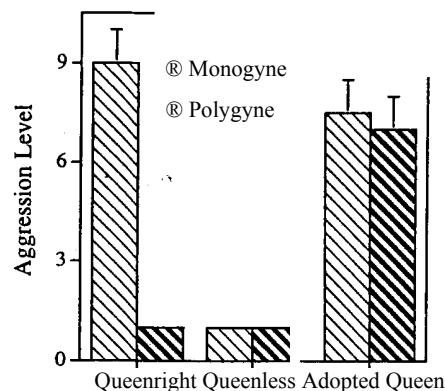


Fig. 2 Plot showing the effect of the adoption of a newly mated queen (NMQ) on conspecific worker nestmate recognition as measured by an aggression bioassay (median and 1/2 IQR shown). Recognition was measured using an aggression bioassay (see Table 1 for the graded aggression response scale). For comparison purposes, the data for queenright and queenless conspecific aggression were taken from the same data used to generate the plot in Fig. 1 (queenright, t=0 days and queenless and broodless, t=35 days). Conspecific aggression scores for monogyne and polygyne queenless colonies that had adopted an NMQ, and monogyne queenright colonies were not significantly different but were significantly higher than their queenless counterpart ($P < 0.05$, see Results).

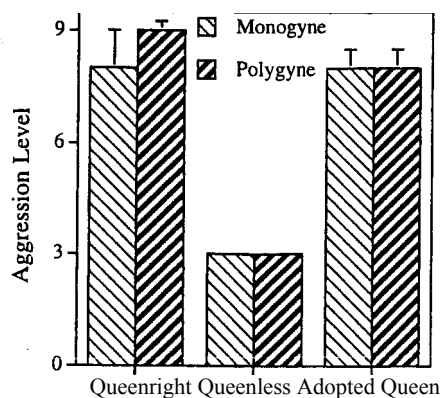


Fig. 3 Plot showing the worker aggression level (median and 1/2 IQR shown) toward NMQ intruders. Three conditions were evaluated for polygyne and monogyne workers: queenright colonies, queenless colonies, and queenless worker groups that had adopted a queen. Recognition was measured using an aggression bioassay (see Table 1 for the graded aggression response scale). None of the queenright and adopted-queen results were significantly different from each other ($P > 0.05$, see Results). Results for queenright and adopted queen were both significantly different from the results for queenless worker groups ($P < 0.05$, see Results)

The influence of the queen on worker aggression toward conspecific NMQs

The colony queen was shown in previous experiments to have a dramatic effect on monogyne worker nestmate recognition. We examined here what influence the presence or absence of the colony queen had on worker aggression toward NMQs. Introduction of NMQ intruders into queenright monogyne colony residents resulted in immediate investigation and eventual alarm and attack. The aggressive behavior of the resident workers was often delayed (bioassays could take up to 30 min) if the NMQ remained motionless upon being contacted (aggression score: median=8.000, IQR=2; see Table 1, Fig. 3). In contrast, NMQs introduced into monogynederived worker groups that had been queenless for >5 weeks elicited significantly less aggressive behavior (median=3.000, IQR=0.000; see Table 1, Fig. 3) from the resident workers than queenright resident workers ($U=9.000$, $P<0.0001$, $n=16$). Queenless workers often aggregated around and walked over the NMQs, sometimes even riding on her back. There was no gaster curling, alarm, or holding down behaviors, typical of higher level aggression. A similar pattern was obtained for the introduction of NMQs into polygyne queenright and queenless colonies (Fig. 3). Queenright polygynederived workers generally accepted monogyne or polygyne-derived workers; however, these polygyne-derived workers were highly aggressive toward NMQs (Fig. 3; aggression score: median=9.000, IQR=0.500; see Table 1; Vander Meer and Porter 2001). In contrast, queenless polygyne workers were significantly less aggressive toward NMQs (Fig. 3; aggression score: median=3.000, IQR=0.000, $n=16$; see Table 1; $U=1.000$, $P<0.0001$).

The effect of an adopted queen on conspecific worker/worker nestmate recognition

Monogyne workers in the absence of their queen were shown to lose their ability to recognize and respond aggressively toward conspecific non-nestmates (Fig. 1), such that aggression levels were only investigative (1-3; Table 1) and significantly lower than the aggression scores of the queenright colonies (see above). The low aggression levels for NMQs introduced into monogyne or polygyne queenless worker groups (Fig. 3) have made it easy to re-queen these groups with NMQs. After adoption of an NMQ by queenless monogyne worker groups, conspecific worker nestmate recognition returned to significantly higher levels (Fig. 2; queenless median=2, IQR=1, $n=18$, vs adopted queenright median=8.000, IQR=0.000; $U=6.000$, $P<0.0001$, $n=18$) that were indistinguishable from those found in naturally occurring queenright monogyne colonies (see Fig. 2; $U=162.000$, $P=0.599$, $n=20$). Polygyne queenless worker groups also readily adopted NMQs. Adoption of one or more NMQs by polygyne queenless groups resulted in a dramatic increase in conspecific worker aggression (Fig. 2; queenless: median=1.000, IQR=0.000, $n=20$ vs with adopted NMQ: median=8.000, IQR=1.25, $n=18$).

The effect of an adopted queen on conspecific worker/newly mated queen aggression

Queenright monogyne and polygyne colonies were highly aggressive toward NMQs (Fig. 3); however, the aggression level for both social forms toward NMQs decreased dramatically after the colonies were made queenless (Fig. 3). With the capability to easily re-queen queenless worker groups, we evaluated the effect of queen adoption on worker aggression toward NMQs. The introduction of NMQs to monogyne-derived queenless worker groups that had adopted a new queen resulted in significantly higher levels of aggression than when NMQs were introduced into queenless monogyne worker groups (Fig. 3; queenless: median=3, IQR=0.000, $n=14$ vs adopted queenright: median=8.000, IQR=1.000, $n=15$; $U=4.500$, $P<0.0001$). The introduction of NMQs into queenless polygyne worker groups that had previously adopted a queen also led to significantly higher levels of worker aggression toward NMQs than when NMQs were introduced into queenless polygyne worker groups (Fig. 3; queenless: median=3.000, IQR=0, $n=16$ vs adopted queenright: median=8.000, IQR=1.000, $n=16$; $U=1.000$, $P<0.0001$). For both monogyne and polygyne adopted-queen worker groups, aggression results were not different from natural queenright colonies (Fig. 3; $P>0.05$).

Discussion

The chemical cue/neural template model of nestmate recognition has focused research toward the cue/template-matching mechanism (Hollnagel and Michener 1980; Hepper 1986; Getz and Chapman 1987; Obin and Vander Meer 1989b), and on the source and chemical nature of the cues (See Breed 1998; Vander Meer and Morel 1998). The cues, environmental and heritable, reside on each individual's cuticle; thus nestmate recognition cue discriminators are derived from all life stages residing in a colony. Allogrooming, trophallaxis, and the postpharyngeal gland have been postulated to provide a mechanism for the facile distribution and mixing of individual discriminators to form a colony "Gestalt" (Crozier and Dix 1979; Crozier 1987; Soroker et al. 1994). Numerous studies have found correlations between cuticular hydrocarbon patterns and nestmate recognition in ants (Breed 1998; Vander Meer and Morel 1998), but few have directly linked purified cuticular hydrocarbons with a nestmate recognition bioassay (Lahav et al. 1999; Thomas et al. 1999). Other nestmate recognition research has focused on attempts to determine the relative importance of recognition discriminators, especially queen-derived discriminators (e.g., Carlin and Hollnagel 1986).

Heritable discriminators produced by *C. floridanus* queens were found to play a central role in nestmate recognition of this species (Carlin and Hollnagel 1983, 1986, 1987). Queen-derived discriminators were found to be of greater importance for nestmate recognition than worker-derived discriminators, or discriminators derived from the environment (Carlin and Hollnagel 1986). Carlin and Hollnagel (1983) proposed that the queen discriminators were distributed and learned by all colony members. Queen-derived discriminators also appear to be important for *Myrmica* species (Brian 1986), *Leptothorax lichtensteini* (Provost 1989), and *Messor barbarus* (Provost et al. 1994). However, in several other species, queen-derived discriminators were determined to not influence nestmate recognition, e.g., *L. curvispinosus* (Stuart 1987), *Rhytidoponera confusa* (Crosland 1990), *Cataglyphis niger* (Lahav et al. 1998), *Cataglyphis iberica* (Dahbi and Lenoir 1998), and most importantly for this study, *S. invicta* (Obin and Vander Meer 1989b).

Our results demonstrating the powerful effect of fire ant queens on conspecific nestmate recognition appear to contradict the earlier study (Obin and Vander Meer 1989b), that showed that queens did not play a role in colony-level recognition. However, that paper focused on determining whether or not queen discriminators -compounds on the surface of the queen - could affect nestmate recognition. All experiments involved the introduction of kin or non-kin workers into queenright colonies. The introduced workers were either queenless or with their mother queen. The presence or absence of the queen was shown to have no effect on the response of the residents in the queenright colonies. Nestmate recognition discriminators represent colony-specific heritable

cues (putatively quantitative rather than qualitative differences; Vander Meer and Morel 1998), and each colony appears different due to phenotypic differences expressed by members of the colony. Obin and Vander Meer (1989b) concluded that queen discriminators did not influence the worker recognition response and that worker discriminators appeared to overwhelm those produced by the queen. While the queen does not contribute significantly to colony-specific discriminators, we have clearly demonstrated here that she dramatically influences conspecific nestmate recognition, presumably through the release of a pheromone rather than colony-specific discriminators. This pheromone does not elicit an immediate, observable releaser pheromone response from recipient workers, instead it acts as a primer pheromone by setting in motion a physiological change in the recipient workers that results in conspecific nestmate recognition (queen present) or lack of conspecific nestmate recognition (queen absent; see Fig. 1). High levels of queenless worker aggression toward heterospecific workers clearly demonstrated that the queen recognition primer pheromone only affects conspecific recognition. It may appear that heterospecific and conspecific recognition are controlled by different mechanisms; however, we propose that the queen recognition primer pheromone modulates (increases) the sensitivity of workers to differences in recognition cues. Thus, without this primer pheromone, workers do not detect the subtle within-species recognition cue differences (quantitative; Vander Meer and Morel 1998), but are capable of detecting the qualitative between-species cue differences. Since there was no discernable decrease in heterospecific aggression, detection sensitivity rather than an aggressive behavior threshold is apparently being modulated. Fire ant recruitment provides precedence for this type of pheromone-controlled increased sensitivity. The orientation inducer pheromone increases the sensitivity (by a factor of 250) of workers to the recruitment orientation pheromone (Vander Meer et al. 1990). Brood can be transferred and adopted from one colony to another, probably due to a combination of chemical and behavioral factors (Vander Meer and Morel 1988). They could have acted as a queen primer pheromone holder or carrier, delaying the decline in conspecific aggression. However, brood did not affect the drop in conspecific aggression after workers were removed from their queen.

How does this queen recognition primer pheromone fit the current nestmate recognition hypothesis for monogyne and polygyne fire ant populations? Under normal queenright conditions, monogyne-derived fire ant workers respond aggressively toward intruders from other colonies. Each colony is insular. Therefore, the experience-based neural template used by a resident worker to compare with the cues of an intruder is narrowly defined. Polygyne populations act as a "unicolony" and workers experience diverse heritable (multiple matrilineal and patrilineal) and environmental cues. The resulting broad neural template tolerates a wide range of heritable and environmental cue profiles, leading to a lack of conspecific

cific worker/worker recognition. This scenario is supported by the observation that when polygyne colonies are brought into the laboratory and do not have the opportunity to interact with other colonies, their aggression levels increase significantly (Obin et al. 1993). The high aggression levels we report from polygyne workers that have adopted an NMQ toward non-nestmate workers could be due to the queen type (monogyne derived), but is most likely caused by their lack of integration into a polygyne population (Obin et al. 1993). Monogyne and polygyne population workers are similarly influenced by the conspecific recognition primer pheromone, except that polygyne worker/worker interactions are affected by the broad template concept discussed above. The high level of aggression elicited by NMQs from queenright polygyne and monogyne workers may be indicative of qualitative as well as quantitative differences in recognition cues. How do these results fit into what is known about fire ant genetics?

Recently a protein-encoding gene, Gp-9, has been reported to be strongly correlated with the two *S. invicta* social forms (Ross and Keller 1998). All monogyne queens, female alates, and workers are BB homozygotes at Gp-9. Functional polygyne queens are always Bb heterozygotes. The bb homozygotes do not survive to the adult stage. Workers and female alates within polygyne colonies can be BB or Bb depending on their father's genotype. Thus, the Gp-9 genotype does not affect the ability of workers and/or female alates to tolerate each other, conforming to the nestmate recognition mechanism outlined above. The majority (80+%) of polygyne-derived female alates mate with monogyne-derived males (Ross 1997) and both female sexual genotypes come down together after their flight. Workers from queenright monogyne colonies execute NMQs of either Gp-9 genotype. Workers from queenright polygyne colonies reject BB NMQs regardless of their origin (monogyne or polygyne). However, in laboratory studies, about 50% of Bb genotype NMQs were executed by queenright polygyne colony workers, and the remaining NMQs were accepted for at least 3 days (Ross and Keller 1998). Why some Bb genotype NMQs were accepted and others executed is unknown.

The NMQs used in our study were collected in a typically monogyne area and were of a weight indicative of monogyne-derived NMQs (Keller and Ross 1993) and must be BB homozygotes at Gp-9 (Ross and Keller 1998). Workers from queenright monogyne and polygyne colonies executed all introduced monogyne-derived NMQs, as expected based on genotype (Ross and Keller 1998) and on behavioral observations (Vander Meer and Porter 2001). However, the conspecific recognition process breaks down soon after the queen or queens are removed. Regardless of the queenless workers' genotype, they are no longer aggressive toward monogyne-derived NMQs. This is contrary to expectations based on genotype (Ross and Keller 1998) and aggression studies with queenright colonies (Vander Meer and Porter 2001) and highlights the remarkable influence monogyne and polygyne queens have on conspecific recognition.

Keller and Ross (1998) suggested that the b allele of the Gp-9 gene was the first example of a "green beard" gene - one that is supposed to give the bearer a discernable external label that makes other "green beard" individuals respond in an advantageous manner. Ideally, polygyne colony workers (Bb) would recognize the presence of the b allele in NMQs (Bb, polygyne derived) and allow them to reproduce, but they would execute NMQs

that do not possess the b allele (BB, monogyne derived). In the laboratory, all BB NMQs and about 50% of the Bb queens were executed (Ross and Keller 1998), thus the ability of polygyne workers to recognize and give preferential treatment to Bb NMQs is borderline. The advantage of the purported "green beard" label dissipates completely among queenless Bb workers, who are non-aggressive toward BB and Bb NMQs (this paper; R.K. Vander Meer and L.E. Alonso, unpublished data). Therefore, the ability of these workers to discriminate between Bb and BB NMQs is not genetically hardwired, but is dependent on the presence or absence of the conspecific recognition primer pheromone that modulates the sensitivity of the worker recognition system toward subtle odor differences. In view of these results the fire ant "green beard" gene argument should be reviewed.

Ideally, the proof of a primer pheromone is the bioassay-driven isolation, identification, and synthesis of active compounds, followed by successful bioassays with synthetic pheromone components. Thus far, no ant primer pheromones have been isolated and identified. Nonpheromone stimuli from the queen, such as egg production or queen/worker physical interactions could explain the results attributed to primer pheromones (Vargo 1998). Queenright *S. invicta* colonies treated with avermectin create a pseudqueenless situation by permanently destroying the queen's ovaries (Glancey et al. 1982). Under these conditions, workers still display strong nestmate recognition capabilities (R.K. Vander Meer, unpublished data); thus, a queen's fecundity is not correlated with the maintenance of conspecific recognition. The dramatic behavioral changes reported here are most readily attributed to a queen-produced primer pheromone. *S. invicta* queens have been shown to produce primer pheromones that (a) influence caste in female larvae and (b) inhibit reproductive activity in other colony members (Vargo 1998). The purported purpose of these primer pheromones is to subdue potential reproductive competition. Similarly, the conspecific recognition primer pheromone we report here increases the probability that newly mated queens dropping into the territory of monogyne or polygyne colonies will be executed, thus preventing reproductive competition for the existing queen or queens. In monogyne colonies, the primer pheromone promotes the maintenance of territoriality and reproductive segregation of individual queens. In polygyne colonies, it limits access of newly mated queens into existing polygyne populations (see Glancey and Lofgren 1988; Porter 1991) to a portion of those of a particular genotype (Ross and Keller 1998) not recognized as being different, thus regulating reproductive competition from

outside the colony. Under this general umbrella of conspecific nestmate recognition is diminished worker/worker recognition in polygyne populations due to the broad template mechanism described above.

This is the first report of a conspecific recognition primer pheromone in social insects; however, it is an addition to an already impressive list of *S. invicta* queen-produced primer pheromones (Vargo 1998). Monogyne and polygyne-derived queens produce primer pheromones that inhibit the production of sexuals (Vargo and Fletcher 1986a, 1986b). Queens pheromonally inhibit competing egg production in alates by preventing dealation (Fletcher and Blum 1983; Fletcher et al. 1983). In polygyne colonies, as the number of queens increases, their individual egg-laying rate decreases (Vander Meer et al. 1992), as a result of each queen producing a primer pheromone that mutually inhibits the egg-laying rate of the other queens (Vargo and Fletcher 1989). A queen releaser pheromone produced by the poison gland and released through the sting apparatus (Vander Meer et al. 1980; Vander Meer and Morel 1995) attracts workers to her for grooming, feeding, and egg removal. This pheromone system (Vander Meer et al. 1980; Vander Meer and Morel 1995) may be central to the distribution of queen-produced primer pheromones (Vander Meer 1983). Future reports will focus on how the conspecific recognition primer pheromone influences NMQ adoption, colony fusion, and colony dynamics in monogyne and polygyne populations.

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References

- Banks WA, Lofgren CS, Jouvenaz D, Stringer CE, Bishop PM, Williams DF, Wojcik DP, Glancey BM (1981) Techniques for collecting, rearing and handling imported fire ants. USDA, SEA-AATS-S-21, pp 1-9
- Breed MD (1998) Nestmate recognition in ants. In: Vander Meer RK, Breed M, Winston M, Espelie KE (eds) Pheromone communication in social insects. Westview, Boulder, Colo, pp 79-103
- Breed MD, Bennett B (1987) Kin recognition in highly social insects. In: Fletcher DJC, Michener CD (eds) Kin recognition in animals. Wiley, New York, pp 243-286
- Brian MV (1986) Bonding between workers and queens in the ant genus *Myrmica*. Anim Behav 34:1135-1145
- Carlin NF, Holldobler B (1983) Nestmate and kin recognition in interspecific mixed colonies of ants. Science 222:1027-1029
- Carlin NF, H61ldobler B (1986) The kin recognition of carpenter ants (*Camponotus spp.*). I. Hierarchical cues in small colonies. Behav Ecol Sociobiol 19:123-134
- Carlin NF, H61ldobler B (1987) The kin recognition system of carpenter ants (*Camponotus spp.*). II. Larger colonies. Behav Ecol Sociobiol 20:209-217
- Crosland MWJ (1990) The influence of the queen, colony size and worker ovarian development on nestmate recognition in the ant *Rhytidoponera confusa*. Anim Behav 39:413-425
- Crozier RH (1987) Genetic aspects of kin recognition: concepts, models, and synthesis. In: Fletcher DJC, Michener CD (eds) Kin recognition in animals. Wiley, New York, pp 55-73
- Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odour in social Hymenoptera. Behav Ecol Sociobiol 4:217-224
- Dahbi A, Lenoir A (1998) Nest separation and the dynamics of the Gestalt odor in the polydomous ant *Cataglyphis iberica* (Hymenoptera, Formicidae). Behav Ecol Sociobiol 42:349-355
- Fletcher DJC, Blum MS (1983) The inhibitory pheromone of queen fire ants: effects of disinhibition on dealation and oviposition by virgin queens. J Comp Physiol A 153:467-475
- Fletcher DJC, Cherix D, Blum MS (1983) Some factors influencing dealation by virgin queen fire ants. Insectes Soc 30:443-454
- Gadagkar R (1985) Kin recognition in social insects and other animals - a review of recent findings and a consideration of their relevance for the theory of kin selection. Proc Indian Acad Sci (Anim Sci) 94:587-621
- Getz WM, Chapman RF (1987) An odor discrimination model with application to kin recognition in social insects. Intern J Neurosci 32:963-978
- Glancey BM, Lofgren CS (1988) Adoption of newly mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* Buren. Fla Entomol 71:581-587
- Glancey BM, Lofgren CS, Williams DF (1982) Avermectin B1a: effects on the ovaries of red imported fire ant queens (Hymenoptera: Formicidae). J Med Entomol 19:743-747
- Greenberg L, Fletcher DJC, Vinson SB (1985) Differences in worker size and mound distribution in monogynous and polygynous colonies of the fire ant *Solenopsis invicta* Buren. J Kansas Entomol Soc 58:9-18
- Halliday RB (1983) Social organization of meat ants *Iridomyrmex purpureus* analyzed by gel electrophoresis of enzymes. Insectes Soc 30:45-56
- Hepper PG (1986) Kin recognition: functions and mechanisms. A review. Biol Rev 61:63-93
- H61ldobler B, Carlin NF (1987) Anonymity and specificity in the chemical communication signals of social insects. J Comp Physiol A Sens Neural Behav Physiol 161:567-581
- H61ldobler B, Michener CD (1980) Mechanisms of identification and discrimination in social Hymenoptera. In: Markl H (ed) Evolution of social behavior: hypothesis and empirical tests. Verlag Chemie, Weinheim, pp 35-58
- H61ldobler B, Wilson EO (1977) The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8-15
- Holmes WG, Sherman PW (1982) The ontogeny of kin recognition in two species of ground squirrels. Am Zool 27:491-517
- Jutsum AR, Saunders TS, Cherrett JM (1979) Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. Anim Behav 27:839-844
- Keller L, Ross KG (1993) Phenotypic basis of reproductive success in a social insect: genetic and social determinants. Science 260:1107-1110
- Keller L, Ross KG (1995) Gene by environment interaction: effects of a single gene and social environment on reproductive phenotypes of fire ant queens. Funct Ecol 9:667-676
- Keller L, Ross KG (1998) Selfish genes: a green beard in the red fire ant. Nature 394:573-575
- Lahav S, Soroker V, Vander Meer RK, Hefetz A (1998) Nestmate recognition in the ant *Cataglyphis niger*: do queens matter? Behav Ecol Sociobiol 43:203-212
- Lahav S, Soroker V, Vander Meer RK, Hefetz A (1999) Direct behavioral evidence for hydrocarbons as ant recognition discriminators. Naturwissenschaften 86:246-249
- Mintzer A (1982) Nestmate recognition and incompatibility between colonies of the acacia-ant *Pseudomyrmex ferruginea*. Behav Ecol Sociobiol 10:165-168
- Morel L, Vander Meer RK, Lofgren CS (1990) Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (Hymenoptera: Formicidae). Ann Entomol Soc Am 83:642-647
- Obin MS (1986) Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae): effect of environment and the role of cuticular hydrocarbons. J Chem Ecol 12:1965-1975

- Obin MS, Vander Meer RK (1988) Sources of nestmate recognition in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Anim Behav* 36:1361-1370
- Obin MS, Vander Meer RK (1989a) Mechanism of template-label matching in fire ant, *Solenopsis invicta* Buren, nestmate recognition. *Anim Behav* 38:430-435
- Obin MS, Vander Meer RK (1989b) Nestmate recognition in fire ants (*Solenopsis invicta* Buren): do queens label workers? *Ethology* 80:25-264
- Obin MS, Morel L, Vander Meer RK (1993) Unexpected, well-developed nestmate recognition in laboratory colonies of polygyne imported fire ants (Hymenoptera: Formicidae). *J Insect Behav* 6:579-589
- Porter SD (1991) Origins of new queens in polygyne red imported fire ant colonies (Hymenoptera: Formicidae). *J Entomol Sci* 26:474-478
- Provost E (1989) Social environmental factors influencing mutual recognition of individual in the ant *Leptothorax lichtensteini* Bondr. (Hymenoptera: Formicidae). *Behav Process* 18:35-39
- Provost E, Riviere G, Roux M, Bagneres, A-G, Clement JL (1994) Cuticular hydrocarbons whereby *Messor barbatus* ant workers putatively discriminate between monogynous and polygynous colonies: are workers labeled by queens? *J Chem Ecol* 20:2985-3003
- Ross KG (1997) Multilocus evolution in fire ants: effects of selection, gene flow and recombination. *Genetics* 145:961-974
- Ross KG, Keller L (1998) Genetic control of social organization in an ant. *Proc Natl Acad Sci* 95:14232-14237
- Ross KG, Vander Meer RK, Fletcher DJC, Vargo E (1987) Biochemical phenotypic and genetic studies of two introduced fire ants and their hybrid (Hymenoptera: Formicidae). *Evolution* 41:283-291
- Sokal RR, Rohlf FJ (1988) *Biometry*. Freeman, San Francisco
- Soroker V, Vienne C, Nowbahari E, Hefetz A (1994) The postpharyngeal gland as a "Gestalt" organ for nestmate recognition in the ant *Cataglyphis niger*. *Naturwissenschaften* 81:510-513
- Stuart RJ (1987) Transient nestmate recognition cues contribute to a multicolonial population structure in the ant, *Leptothorax curvispinosus*. *Behav Ecol Sociobiol* 21:229-235
- Thomas ML, Parry LJ, Allan RA, Elgar MA (1999) Geographic affinity, cuticular hydrocarbons and colony recognition in the Australian meat ant *Iridomyrmex purpureus*. *Naturwissenschaften* 86:87-92
- Traniello JFA (1982) Population structure and social organization in the primitive ant *Amblyopone nallines* (Hymenoptera: Formicidae). *Psyche* 89:65-80
- Vander Meer RK (1983) Semiochemicals and the red imported fire ant (*Solenopsis invicta* Buren) (Hymenoptera: Formicidae). *Fla Entomol* 66:13-161
- Vander Meer RK, Morel L (1988) Brood pheromones in ants. In: Trager JC (ed) *Advances in myrmecology*. Brill, New York. pp 491-513
- Vander Meer RK, Morel L (1995) Ant queens deposit pheromones and antimicrobial agents on eggs. *Naturwissenschaften* 82:93-95
- Vander Meer RK, Morel L (1998) Nestmate recognition in ants. In: Vander Meer RK, Breed M, Winston M, Espelie KE (eds) *Pheromone communication in social insects*. Westview, Boulder, Colo, pp 79-103
- Vander Meer RK, Porter SD (2001) Fate of newly mated queens introduced into monogyne and polygyne fire ant colonies. *Ann Entomol Soc Am* 94:289-297
- Vander Meer RK, Glancey BM, Lofgren CS, Glover A, Tumlinson JH, Rocca J (1980) The poison sac of red imported fire ant queens: source of a pheromone attractant. *Ann Entomol Soc Am* 73:609-612
- Vander Meer RK, Lofgren CS, Alvarez FM (1985) Biochemical evidence for hybridization in fire ants. *Fla Entomol* 68:501-506
- Vander Meer RK, Saliwanchik D, Lavine B (1989) Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*: implications for nestmate recognition. *J Chem Ecol* 15:2115-2125
- Vander Meer RK, Lofgren CS, Alvarez FM (1990) The orientation inducer pheromone of the fire ant *Solenopsis invicta*. *Physiol Entomol* 15:483-488
- Vander Meer RK, Morel L, Lofgren CS (1992) A comparison of queen oviposition rates from monogyne and polygyne fire ant, *Solenopsis invicta*, colonies. *Physiol Entomol* 17:384-390
- Vargo EL (1998) Primer pheromones in ants. In Vander Meer RK, Breed M, Winston M, Espelie KE (eds) *Pheromone communication in social insects*. Westview, Boulder, Colo, pp 293-313
- Vargo EL, Fletcher DJC (1986a) Evidence of pheromonal queen control over the production of male and female sexuals in the fire ant, *Solenopsis invicta*. *J Comp Physiol A* 159:741-749
- Vargo EL, Fletcher DJC (1986b) Queen number and the production of sexuals in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 19:417
- Vargo EL, Fletcher DJC (1989) On the relationship between queen number and fecundity in polygyne colonies of the fire ant *Solenopsis invicta*. *Physiol Entomol* 14:223-232
- Wilson EO (1971) *The insect societies*. Belknap, Cambridge, Mass